110> APPLICANT: KUWABARA, HASHIGUCHI, Kenichi NAKAMATSU, Tsuyoshi KURAHASHI, Osamu MORI, Yukiko ITO, Hisao 120> TITLE OF INVENTION: CARBAMOYL-PHOSPHATE SYNTHETASE GENE OF CORYNEFORM BACTERIA AND METHOD FOR PRODUCING L-ARGININE 130> FILE REFERENCE: OP945CIP 140> CURRENT APPLICATION NUMBER: US/09/629,616 141> CURRENT FILING DATE: 2000-07-31 160> NUMBER OF SEQ ID NOS: 7 170> SOFTWARE: PatentIn Ver. 2.0 210> SEQ ID NO 1 211> LENGTH: 4837 212> TYPE: DNA 213> ORGANISM: Brevibacterium lactofermentum 220> FEATURE: 221> NAME/KEY: CDS 222> LOCATION: (283)..(1461) 221> NAME/KEY: CDS 222> LOCATION: (1470)..(4808) 400> SEQUENCE: 1 gatccaggaa aaacctggac agcatccggt gcagactttg cgtccaaggo tgaaaacacc 60 ccatttgagg gccaggaatt cagcgctaag gtcacacaca ccgtgcttcg tggcaaggtg 120 acttgtgcag acggagttgc gcaagacgct taacgggtgg gtgcatagta tgcacgcgcc 180 gcattgcata taatgcaatg aattgaataa actagattca gggttatcaa ccagccaatt 240 tcttttaaaa agacagacac acgaaaggcg acaaccagtca cc gtg agt aaa gac Val Ser Lys Asp acc acc acc tac cag gga gtc acc gag atc gga tcc gtt ccg gca tac Thr Thr Thr Tyr Gln Gly Val Thr Glu Ile Gly Ser Val Pro Ala Tyr 15 10 ctg gtt ctt gca gac gga cgt agc ttc acc gga ttt ggc ttt gga gct 390 Leu Val Leu Ala Asp Gly Arg Thr Phe Thr Gly Phe Gly Phe Gly Ala 30 25 atc ggc acc acc ctt ggt gag gca gtg ttc acc acc gcc atg acc ggt Ile Gly Thr Thr Leu Gly Glu Ala Val Phe Thr Thr Ala Met Thr Gly 438 45 40 tac caa gaa acc atg acc gat cct tcc tat cac cgc cag att gtt gtg 486 Tyr Gln Glu Thr Met Thr Asp Pro Ser Tyr His Arg Gln Ile Val Val 60 55 get acc gca cca cag aftc ggt aac acc ggc tgg aac gat gag gac aac 534 Ala Thr Ala Pro Gln #le Gly Asn Thr Gly Trp Asn Asp Glu Asp Asn 75 gag too ogo gac gg $ot\!\!\!/$ aag att tgg gtt gca ggo ott gtt ato ogo gac 582 Glu Ser Arg Asp Gy Lys Ile Trp Val Ala Gly Leu Val Ile Arg Asp 95 90 ctc gca gca cgt/gtg tcc aac tgg cgc gcc acc acc tcc ttg cag cag 630 Leu Ala Ala Arg Val Ser Asn Trp Arg Ala Thr Thr Ser Leu Gln Gln 115 110 gaa atg gca gac caa ggc atc gtc ggc atc ggc gga atc gac acc cgc 678 Glu Met Ala Asp Gln Gly Ile Val Gly Ile Gly Gly Ile Asp Thr Arg 130 125 120 gca ctg gt# cgc cac ctg cgc aac gaa ggt tcc atc gca gcg ggc atc 726 Ala Leu Vạn Arg His Leu Arg Asn Glu Gly Ser Ile Ala Ala Gly Ile 145 140 774 ttc tcc ggc gct gac gca cag cgc cca gtt gaa gaa ctc gta gag atc Phe Ser/Gly Ala Asp Ala Gln Arg Pro Val Glu Glu Leu Val Glu Ile 160 155 gtc aag aat cag cca gca atg acc ggc gca aac ctc tcc gtt gag gtc Val Lys Asn Gln Pro Ala Met Thr Gly Ala Asn Leu Ser Val Glu Val 175 170 tot get gat gaa ace tac gtc atc gaa get gag ggc gaa gag egc cac 870

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Ser Ala Asp Glu Thr Tyr Val Ile Glu Ala Glu Gly Glu Glu Arg His

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αаа	gtt	ctg	gaa	gcc	gac	att	cca	ttc	ttt	ggc	atc	tgc	ttc	ggc	aa	ac	1110
Glu	Val	Leu	Glu	Ala 265	Asp	Ile	Pro	Phe	Phe 270	Gly	Ile	Cys	Pne	275	AS	511	1150
cag	atc	ctc	ggc	cgc	gca	ttc	ggc	atg	gag	acc Thr	tac	aag	-ctg	Lvs	Pł	ne	1158
Gln	Ile	Leu	280	Arg	Ala	Pne	GIY	Met 285	GIU	1111	7	- uys	290				
aac	cac	cgc	aac	atc	aac	gtt	cca	gtg	aag	aag	cac	ato	acc	ggc	aa	ag	1206
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				345				/	350					30:	>		1398
gca Ala	tac Tyr	tcc Ser	Val	Gln	tac Tyr	cac His	CCA Pro	gag Glu	gcc Ala	Ala	gco Ala	ggo a Gly	y Pro	O ASI	n A	sp	1330
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Ala	Sei	Pro 375	Leu	Phe	Asp	Gln	Phe 380	Vai	Glu	Lev	ı Me	As ₁	D AT	a As	ρA	la	
cac	aag	g aaa	, ggc	gca	taa	at/aa	c at	g cc	a aa	ıg cç	gt t	ca g	at a	tt a	ac	cac	1496
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gto	cto	gto	ato	ggt	tcc	ggc	ccc	atc	gto	att	gg Cl	c ca	g gc	a tg	t g	gaa 3111	1544
Va:		ı Val	Ile	e GTA	/ Ser /15	GIY		Ile		20	9 GI	y Gi	II AI	асу	5	25	
++	- ca	c tac	tco	ggo	:/acc	cao	gct	tgc	cgo	gt	g ct	g aa	g ga	a ga	g g	gga	1592
Ph	e As	р Туз	s Sei	c Gly 30	/ Thr	Gln	ı Ala	ı Cys	Arg	g Va. 5	т ге	u Ly	S GI	.u G1	.u (эту	
ct	g cg	c gt	aco	c g/to	ato	aac	tcc	aac	CC	a gc	a ac	g at	c at	g ac	c g	gac	1640
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ca	ıg ct	.g/ ga	t cg	c ct	c gg	c at	c ct	g gaa	a aa	g ta	c g	ge g	tt g	aa c lu t	tc en	atc	1832
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	cca	ata	qtc	gtg	cgt	сса	tcc	ttc	act a	atg (ggt	ggc	ctg	ggc	tcc	ggj	2024
	Pro	Val	Val	Val	Arg	Pro	Ser	Phe	Thr !	Met (Gly	Gly	Leu	Gly	Ser	∮ 1y	
	170					175					180					/185	2072
	ctt	gca	tac	aac	acc	gaa	gac	ctt	gag	cgc	atc Tlo	gca	ggt	ggc	gga	T.en	2072
	Leu	Ala	Tyr	Asn	190	GIU	ASP	ьeu	Glu .	195	116	AIA	GIY	Gry	200	БСС	
	act	gca	tct	cct		qca	aac	gtc	ttg		gaa	gaa	tcc	atc/	ctt	ggt	2120
	Ala	Ala	Ser	Pro	Glu	Āla	Asn	Val	Leu	Ile	Glu	Glu	Ser	Ιλ	Leu	Gly	
				205					210					2/15			2160
	tgg	aag	gaa	ttc	gag	ctc	gag	ctc	atg	cgc	gat	acc	gca	/gac	aac	gtt Val	2168
	Trp	Lys	220	Pne	GIU	ьeu	GIU	225	Met	Arg	АБР	1111	230	АЗР	AJII	Val	
	ata	att		tac	tcc	att	gaa		gtc	gac	gça	ctg	ggc	gtg	cac	acc	2216
	Val	Val	Ile	Cys	Ser	Ile	Glu	Asn	Val	Asp	Ala	Leu	/G2/g	Val	His	Thr	
		235					240					245			/	~~~	2264
	ggc	gac	tct	gtc	acc	gtg	gca	cct	gcc	ctg	acc	ZZ g	act Thr	gac	/cgt	Glu	2264
	Gly 250	Asp	Ser	Val	Thr	255	Ата	Pro	Ala	Leu	260	Leu	1111	7	Arg	265	
	250 ttc	cad	aaσ	ato	cac		caq	aat	atc	gcc/	ate	atc	cgc	gag	gtc	g.g.c	2312
	Phe	Gln	Lys	Met	Arg	Asp	Gln	Gly	Ile	Ala	I/e	Ile	Arg	Glu	Val	Gly	
					270					2 7 5	/				280		2260
	gtg	gac	acc	ggt	gga	tgt	aac	atc	cag	ttc/	gct	ate	-áac	cca	gtt	gat	2360
	Val	Asp	Thr	Gly 285		Cys	Asn	iie	Gln 290	Pro	Ara	тте	ASII	295	, vai	ASP	
	~~~	cac	ato	∠85 atc	acc	att	aaa	ato	aac	cca	cat	ata	tct			tcc	2408
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	gcg	ctg	gca	tcc	aag	gca	acg	ggc	ttc	cca	att	gcc	aag	ato	gct	gcc	2456
	Ala			. Ser	Lys	Ala		GIY	Phe	Pro	тте	A1a 325	Съуз	Met	. Ala	Ala	
	224	315	r act	· ato	. aas	tac	320 acc	cta	/gat	αaα	atc			gac	ato	act	2504
	Lvs	Leu	ı Ala	i Ile	Gly	Tyr	Thr	Leu	Asp	Glu	Ile	Thr	Asn	Āsp	ıle	Thr	
	330					335		/			340					345	2552
	ggt	gaa	acc	c cca	gct	gcg	ttt	gag	ccc	acc	atc	gac	tac	gto	gto	g gtc	2552
	Gly	Glı	ı Thi	r Pro	350 350		Phe /	/ GIu	Pro	355	TIE	ASL	) TĂT	. va.	360	l Val	
	220			a caa			trc	gag	aaq		ato	ggg	get	gat		act	2600
	Lvs	Ala	a Pro	o Arg	g Phe	Ala	Phe	Glu	Lys	Phe	Val	. Gly	y Ala	a As	o Ası	o Thr	
	_			365	5		/		370					37	5		2640
	ttg	aco	e acc	c acc	ato	g aag	tcc	gto	ggt	gag	gto	ato	g tco	c Cty	g ggo	c cgt	2648
	Leu	Th	r Th: 38		r Met	. PAs	Ser	. vai 385	. Сту	GIU	vai	. Me	390	) r ne	u Gi	y Arg	
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	Ala	a Th	r As	p Ly	s Al	a Ala	a Val	l Lei	ı Glu	ı As <u>r</u>	Lei	u Ly	s Ar	g Pr	o In	r Giu	l
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	ggo	c cg	c ct	c/ta	c ga	c gt	t gag	g ct	g gca	ato	g cg	c ct	t gg	c go	a ag	c gtg	2840
	Gly	y Ar	g Le	u/ Ty 44		p va.	I GII	и ге	u Ala 450	i Mei	_ AI	g ne	u Gi	45	55	er Val	•
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	ct	t ga	a/go	t ct	c gt	g ca	g tt	c ca	c cag	g aa	g ct	c gt	t ga	c go	ca co	ca tto	2936
	Le	u Gl 47		a Le	eu Va	1 G1:	n Ph 48		g G11	т гу	s re	u Vā 48	ат АS 35	νp Α.	La PI	co Phe	-
	ct	c aá	ic as	aa da	it at	a at	a ca	c qa	a qc	a aa	g tt	c at	g gg	gt ct	tg to	cc gad	2984
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	cgc Arg	acc Thr	ttg Leu	Arg	ctg Leu	tcc Ser	cta Leu	ggc Gly	Ile	cgc Arg	cca Pro	gta Val	ttc Phe	Lys	act Thr	gtg Væll	<b>.</b>	3080
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	gca Ala	Tyr	gag Glu	ctg Leu	gat Asp	cca Pro	gca Ala 560	gct Ala	gag Glu	tct Ser	gag Glu	gtc Val 565	gca Ala	cca Pro	cag Gln	act Thr	: :	3176
	gag Glu	555 cgt Arg	gaa Glu	aag Lys	gtc Val	ctg Leu	atc	ttg Leu	ggc Gly	tcc Ser	ggt Gly	cca	aac Asn	ggc Arg	atc Ile	Gly	7	3224
	570					575					580					585		2070
	cag Gln	ggc Gly	atc Ile	gag Glu	ttc Phe 590	gac Asp	tat Tyr	tcc Ser	tgt Cys	gtt Val 595	cac His	gca Ala	ggt Ala	ctt Leu	gag Glu 600	Lei	2 1	3272
	tcc Ser	cgc Arg	gtc Val	Gly	tac Tyr	gaa Glu	act Thr	gtc Val	Met	gtc	aac Asn	tg¢ Cys	aac Asn	Pro	Glu	acc Th:	e r	3320
	gtg Val	tcc Ser	acc Thr	605 gac Asp	tac Tyr	gac Asp	acc Thr	gct Ala	610 gac Asp	cgc Arg	ctg Ley	tac Tyr	\ttc Phe	615 gag Glu	сса	ct;	g u	3368
	acc	ttc	620 gaa	gac	gtc	atg	gag	625 gtc	tac	çac	gct	gag	gcg 530	cag	tcc	gg	С	3416
	acc	635 atc	gca	aat	Val gtt	atc	640 gtc	cag	gtt	gg/t	, ggc	645 cag	act	cct	ctg	ı gg	С	3464
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	Leu	Ala	Asp	Arg	Leu 670	Lys	Lys	Ala		√Val 675	Pro	Val	. 11∈	e GTZ	7 Thi 680	r Se	r	
	cca Pro	gag Glu	gca Ala	atc Ile 685	gac Asp	atg Met	gct Ala	gag Glu	gac Asp 690	cgt Arg	ggc	gag Glu	ttc Phe	: ggt : Gl : 695	, Ala	act aLe	g :u	3560
	ctg Leu	aac Asn	Arg	Glu	cag Gln	ctt Leu	cct Pro	ggt Ala 705	Pro	gca Ala	ttc Phe	Gl?	aco Thr	: Ala	a aco	t to	t	3608
	ttc	caa	700	ı act	. cgc	aca	ata			gac	ato	ago			a qt	g ct	g	3656
	Phe	Glu 715	Glu	ı Ala	Arg	Thr	Va/1 7/20	Ala	Asp	Glu	ıIl∈	Ser 725	: Туі 5	r Pro	o Va	l L∈	eu	2504
	gtt Val 730	Arg	cct Pro	tcc Ser	tac Tyr	gtc Val 735	/Leu	ggt Gly	ggc Gly	cgt Arg	ggc Gl _y 740	, Met	g gag Glu	y at u Il	t gt e Va	1 T3	ac /r 15	3704
	gat	gac	g gct 1 Ala	t tcc a Ser	ctc Leu	ga⁄g GAu	gat	tac Tyr	atc Ile	aac Asr 759	ı Arç	g gca	a act	t ga r Gl	g tt u Le 76	u Se	et er	3752
	tct Ser	gac Asr	c cac	c cca s Pro	750 a gtg val	/ctg	gtt Val	gac L Asp	Arg	tto Phe	cto	g ga ı As	c aa p As	n Al	t at a Il	t ga	ag lu	3800
	atc	gad	c gto	765 gad 1 Ast	o gga pAla	ctg Leu	g tgo ı Cvs	c gad s Asr	770 ggc Glv	gad	c gaa	a gt u Va	c ta l Ty	77 c ct r Le	g gc	g g a G	gc ly	3848
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	ggc Gly	gtc Val	aac	ctg Leu	gcc Ala	aag Lys	Ala	gca	tcc Ser	cgt Arg	atc Ile	Ala	gtg	ggc Gly	gcc Ala	acc Thr	42/36
	atc Ile	875 aag Lys	gat Asp	ctc Leu	caa Gln	gat Asp	880 gag Glu	ggc Gly	atg Met	att Ile	cct Pro	885 acc Thr	gag Glu	tac Tyr	gac Asp	Gl⁄⁄	4184
	890 ggc Glv	tcc Ser	ttg Leu	cca Pro	ctg Leu	895 gac Asp	gct Ala	cca Pro	atc Ile	gct Ala	900 gtg Val	aag Lys	gaa Glu	gca Ala	gtg Vaļ⁄	905 ttg Leu	4232
	ccg	ttc Phe	aac	cgc	910 ttc	cgt	cgc	cca	gat	915 gga	aag	acc	ctg	gac	92/0 acc	ctg	4280
	ctt	tcc Ser	cca	925 gag	atg	aag	tcc	act	930 ggc	gag	gtc	atg	ggc	935 trg	gcc	aac	4328
	aac	ttc	940 ggc	gct	gca	tat	gca	945 aag	gct	gaa	gct	ggc	950 gcg	ttt	ggt	gca	4376
		Phe 955 cca	_				960					965			\	Ala aag	4424
	Leu 970	Pro	Thr	Glu	Gly	Thr 975	Val	Phe	Val	Thr	Val 980	A	Asn	Arg	Asp	Lys 985 aag	4472
	Arg	Thr	Leu	Ile	Leu 990	Pro	Ile	Gln	Arg	Leu 995	Ala	/Ser	Met	G <b>/</b> Y	Tyr 1000	Lys	
	atc Ile	ctc Leu	Ala	acc Thr 1005	gaa Glu	ggc Gly	acc Thr	Ala	ggc Gly 1010	M∉t	ctg Leu	Arg	cgc Arg	Aac Asn 1015	ggc	att Ile	4520
	gat Asp	Cys	gaa Glu 1020	Val	gtg Val	ctc Leu	Lys	gct Ala 1025	Ser	ga¢ Asp	atc Ile	cgc Arg	gaa Glu 1030	. Gly	gta Val	gag Glu	4568
	Gly	aag	tcc Ser	atc	gtg Val	Asp	cgt Arg 1040	Ile	cgc Arg	/gaa Glu	ggc	gaa Glu 1045	Val	gac Asp	ctc Leu	atc lle	4616
	ctc Leu	aac Asn	acc	cca Pro	Ala	ggt Gly	tct Ser	gct	ggc Gly	gct Ala	cgc Arg	cac His	gat	ggc Gly	tac Tyr	gat Asp 1065	4664
	105 atc Ile	cgc	gca Ala	gca Ala	gca Ala	Val	acc	gt/g Val	/ ggt Gly	Val	cca Pro	ctg	ato Ile	acc Thr	Thi	gtc Val	4712
	cag Glr	ggt Gly	gtc Val	acc Thr	1070 gca Ala	gct	gtc Va/	caç Glr	ı Gly	, Il∈	gaç	g gco ı Ala	c cto	ı Arç	, Gli	r GJA g agc	4760
	gtt Val	gtc Val	ago Ser	1085 gtc Val	cgc	gcg Ala	g øtg Let	caç Glr	1090 g gaa n Glu	cto	gac Asp	c cac	gca s Ala	1095 a gto a Val	aag	g gct s Ala	4808
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			3	5 /				4	0				4	5		r Thr	
		50 n Ile	0	/			5! r Ala	5			e Gl	6	0			p Asn 80	
	•	_	/														

Asp Glu Asp Asn Glu Ser Ang Asp Gly Lys Ile Trp Val Ala Gly Leu 90 · Val Ile Arg Asp Leu Ala Ala Arg Val Ser Asn Trp Arg Ala Thr Thr 100 105 Ser Leu Gln Gln Glu Met Ala Asp Gln Gly Ile Val Gly Ile Gly Gly 120 Ile Asp Thr Arg Ala Leu Val Arg His Leu Arg Asn Glu Gly Ser Il 135 140 Ala Ala Gly Ile Phe Ser Gly Ala Asp Ala Gln Arg Pro Val Glu 💋 Lu 150 155 Leu Val Glu Ile Val Lys Asn Gln Pro Ala Met Thr Gly Ala AsA Leu 165 170 Ser Val Glu Val Ser Ala Asp Glu Thr Tyr Val Ile Glu Ala Álu Gly 185 180 Glu Glu Arg His Thr Val Val Ala Tyr Asp Leu Gly Ile Lys Gln Asn 195 200 205 Thr Pro Arg Arg Phe Ser Ala Arg Gly Val Arg Thr Val /Ile Val Pro 215 220 Ala Glu Thr Pro Leu Glu Asp Ile Lys Gln Tyr Asn Pro Ser Gly Val 230 235 Phe Ile Ser Asn Gly Pro Gly Asp Pro Ala Ala Ala Asp Val Met Val 250 245 Asp Ile Val Arg Glu Val Leu Glu Ala Asp Ile / Pr/o Phe Ph/e Gly Ile 260 265 Cys Phe Gly Asn Gln Ile Leu Gly Arg Ala Phe Gly Met Glu Thr Tyr 285 280 Lys Leu Lys Phe Gly His Arg Gly Ile Asn Val Pro Val Lys Asn His 295 Asm His Gly Phe Ala Leu Ile Thr Gly Lys Ile Asp Ile Thr Ala Gla 315 310 Lys Gly Glu Ala Gly Gln Glu Phe Glu Thr Asp Phe Gly Thr Ala Ile 32/0 325 Val Thr His Thr Cys Leu Asn Asp Gly ∜al Val Glu Gly Val Ala Leu 340 345 Lys Ser Gly Arg Ala Tyr Ser Val Glr Tyr His Pro Glu Ala Ala Ala 360 Gly Pro Asn Asp Ala Ser Pro Leu Phe Asp Gln Phe Val Glu Leu Met 375 Asp Ala Asp Ala Gln Lys Lys Gly Ala 390

<210> SEQ ID NO 3 <211> LENGTH: 1113

<212> TYPE: PRT

<213> ORGANISM: Brevibacterium /actofermentum

<400> SEQUENCE: 3

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His Glu Thr Val Ala Glu beu Gly Leu Pro Val Val Val Arg Pro Ser - Phe Thr Met Gly Gly Leu Gly Ser Gly Leu Ala Tyr Asn Thr Glu Asp Leu Glu Arg Ile Ala Gly Gly Gly Leu Ala Ala Ser Pro Glu Ala Asn Val Leu Ile Glu Glu Ser Ile Leu Gly Trp Lys Glu Phe Glu Leu Glu Leu Met Arg Asp Thr Ala Asp Asn Val Val Ile Cys Ser Ile Gly Asn Val Asp Ala Leu Gly Val His Thr Gly Asp Ser Val Thr Val Ala Pro Ala Leu Thr Leu Thr Asp Arg Glu Phe Gln Lys Met Arg Asp Gln Gly Ile Ala Ile Ile Arg Glu Val Gly Val Asp Thr Gly Gly  $\nearrow$ cys Asn Ile Gln Phe Ala Ile Asn Pro Val Asp Gly Arg Ile Ile Thr Ile Glu Met Asn Pro Arg Val Ser Arg Ser Ser Ala Leu Ala Ser/Lys Ala Thr Gly Phe Pro Ile Ala Lys Met Ala Ala Lys Leu Ala Ile Gly Tyr Thr Leu Asp Glu Ile Thr Asn Asp Ile Thr Gly Glu Thy Pro Ala Ala Phe Glu Pro Thr Ile Asp Tyr Val Val Val Lys Ala Pro Arg Phe Ala Phe Glu Lys Phe Val Gly Ala Asp Asp Thr Leu Thr/Thr Thr Met Lys Ser Val Gly Glu Val Met Ser Leu Gly Arg Asn Tyr Ile Ala Ala Leu Asn 95 Lys Ala Leu Arg Ser Leu Glu Thr Lys Gln Gly Phe Trp Thr Lys 41Ø Pro Asp Glu Phe Phe Ala Gly Glu Arg A/2a Thr Asp Lys Ala Ala Val Leu Glu Asp Leu Lys Arg Pro Thr Glu/Gly Arg Leu Tyr Asp Val Glu Leu Ala Met Arg Leu Gly Ala Ser Va/l Glu Glu Leu Tyr Glu Ala Ser Ser Ile Asp Pro Trp Phe Leu Ala Glu Leu Glu Ala Leu Val Gln Phe Arg Gln Lys Leu Val Asp Ala Pro Phe Leu Asn Glu Asp Leu Leu Arg Glu Ala Lys Phe Met Gly Leu Ser Asp Leu Gln Ile Ala Ala Leu Arg Pro Glu Phe Ala Gly Glu Asp Gly Val Arg Thr Leu Arg Leu Ser Leu Gly Ile Arg Pro Val Phe Lys Thr Val Asp Thr Cys Ala Ala Glu Phe Glu Ala Lys Thr Pro Tyr His Tyr Ser Ala Tyr Glu Leu Asp Pro Ala Ala Glu Ser Glu Val Afía Pro Gln Thr Glu Arg Glu Lys Val Leu Ile Leu Gly Ser Gly Pro/Asn Arg Ile Gly Gln Gly Ile Glu Phe Asp Tyr Ser Cys Val His Ala Leu Glu Leu Ser Arg Val Gly Tyr Glu Thr Val Met Val Asn  $\not{
m C}$ ys Asn Pro Glu Thr Val Ser Thr Asp Tyr Asp Thr Ala Asp Arg Lev Tyr Phe Glu Pro Leu Thr Phe Glu Asp Val Met Glu Val Tyr His Ala Glu Ala Gln Ser Gly Thr Val Ala Gly Val Ile Val Gln Leu Gly/Gly Gln Thr Pro Leu Gly Leu Ala Asp Arg Leu Lys Lys Ala Gly Va/ Pro Val Ile Gly Thr Ser Pro Glu Ala Ile Asp Met Ala 

Glu Asp Arg Gly Glu Phe Gry Ala Leu Leu Asn Arg Glu Gln Leu Pro Ala Pro Ala Phe Gly Thr Ala Thr Ser Phe Glu Glu Ala Arg Thr Val 710 Ala Asp Glu Ile Ser Tyr Pro Val Leu Val Arg Pro Ser Tyr Val Leu 725 730 Gly Gly Arg Gly Met Glu Ile Val Tyr Asp Glu Ala Ser Leu Glu Asp 740 745 Tyr Ile Asn Arg Ala Thr Glu Leu Ser Ser Asp His Pro Val Leu **Y**al 765 760 755 Asp Arg Phe Leu Asp Asn Ala Ile Glu Ile Asp Val Asp Ala Ley Cys 775 770 780 Asp Gly Asp Glu Val Tyr Leu Ala Gly Val Met Glu His Ile ှ u Glu 790 795 Ala Gly Ile His Ser Gly Asp Ser Ala Cys Ala Leu Pro Pro/Met Thr 810 Leu Gly Ala Gln Asp Ile Glu Lys Val Arg Glu Ala Thr Lys Lys Leu 820 825 Ala Leu Gly Ile Gly Val Gln Gly Leu Met Asn Val Gln/Tyr Ala Leu 840 Lys Asp Asp Ile Leu Tyr Val Ile Glu Ala Asn Pro Ard Ala Ser Arg 855 860 Thr Val Pro Phe Val Ser Lys Ala Thr Gly Val Asn Ley Ala Lys Ala 875 870 Ala Ser Arg Ile Ala Val Gly Ala Thr Ile Lys Asp/Leu Gln Asp Glu 890 885 Gly Met Ile Pro Thr Glu Tyr Asp Gly Gly Ser Leu Pro Leu Asp Ala 900 905 Pro Ile Ala Val Lys Glu Ala Val Leu Pro Phe Asn Arg Phe Arg Arg 925 920 Pro Asp Gly Lys Thr Leu Asp Thr Leu Leu Ser Pro Glu Met Lys Ser 935 940 Thr Gly Glu Val Met Gly Leu Ala Asn Asn Phe Gly Ala Ala Tyr Ala 955 950 Lys Ala Glu Ala Gly Ala Phe Gly Ala Ley Pro Thr Glu Gly Thr Val 965 970 Phe Val Thr Val Ala Asn Arg Asp Lys Arg Thr Leu Ile Leu Pro Ile 990 985 980 Gln Arg Leu Ala Ser Met Gly Tyr Lys/Ile Leu Ala Thr Glu Gly Thr 1000 1005 Ala Gly Met Leu Arg Arg Asn Gly I∤e Asp Cys Glu Val Val Leu Lys 1020 1015 Ala Ser Asp Ile Arg Glu Gly Val Glu Gly Lys Ser Ile Val Asp Arg 1035 1030 Ile Arg Glu Gly Glu Val Asp Ley Ile Leu Asn Thr Pro Ala Gly Ser 1050 1045 Ala Gly Ala Arg His Asp Gly Tyr Asp Ile Arg Ala Ala Ala Val Thr 1070 1060 1065 Val Gly Val Pro Leu Ile Thr∥Thr Val Gln Gly Val Thr Ala Ala Val 1080 1085 1075 Gln Gly Ile Glu Ala Leu A#g Glu Gly Val Val Ser Val Arg Ala Leu 10/95 Gln Glu Leu Asp His Ala #al Lys Ala 1110

<210> SEQ ID NO 4

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificia∜ Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer for amplifying kanamygin resistant gene of Streptococcus faecalis

<400> SEQUENCE: 4

cccgttaact gcttgaaacc caggacaata ac

<210> SEQ ID NO 5 <211> LENGTH: 30 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer for amplifying kanamycin resistant gene of Streptococcus faecalis <400> SEQUENCE: 5 cccgttaaca tgtacttcag aaaagattag 30 <210> SEQ ID NO 6 <211> LENGTH: 26 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer amplifying Escherichia coli cloning vector pHSG399 <400> SEQUENCE: 6 gatatctacg tgccgatcaa cgtctc 26 <210> SEQ ID NO 7 <211> LENGTH: 25 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer for amplifying Escherichia coli cloning vector pHSG399 <400> SEQUENCE: 7 aggccttttt ttaaggcagt tattg 25